COMPOSITION AND ORGANIZATION OF THE *PENICILLIUM* AND ITS TELEOMORPHS TAXOCENE OF TWO GRAZING LAND SOILS IN URUGUAY

Lina BETTUCCI and Claudia RODRÍGUEZ

Departamento de Botánica, Facultad de Humanidades y Ciencias.
Tristán Narvaja 1674. Montevideo. Uruguay.

ABSTRACT - *Penicillium* and its teleomorphs *Eupenicillium* and *Talaromyces* were isolated from the A_1 horizon of 2 grazing land soils at Canelones (Uruguay). The 16 species of those genera form a natural association or taxocene. They represent 57.4% (43.4% at site A and 68.4% at site B) of the 43 372 isolates obtained by the dilution plate analysis at pH 4.5 and 20°C. Four samplings, one at each season, were made during a year. *E. brefeldianum*, the dominant species at site A, was present in all the samples as well as *P. simplicissimum* (with much lower density). At site B, *E. brefeldianum* and *E. shearii* codominate or alternate their dominance according to the month of sampling and were found between 95% and 100% of the samples. At both sites the similarity between the taxocene of each month and the taxocene resulting from the summation of all samples is very high (80%-90%). The organization of both taxocenes based on the arrangement of the abundance and frequency of its components is not altered by the repeated sampling in different seasons.

RÉSUMÉ - Les *Penicillium* et leurs téléomorphes *Eupenicillium* et *Talaromyces* ont été isolés et étudiés à partir de l'horizon A_1 de 2 prairies (Canelones, Uruguay). Les 16 espèces isolées forment une association naturelle ou taxocène. Elles représentent 57.4% (43,4% dans le site A et 68,4% dans le site B) des 43 372 isolements obtenus par l'analyse de dilution du sol, sur le milieu de culture. On a effectué 4 échantillonnages saisonniers pendant une année. *E. brefeldianum*, l'espèce dominante dans le site A, a été isolée dans tous les échantillons prelevés, *P. simplicissimum* également mais avec une densité beaucoup plus faible. Au site B, *E. brefeldianum* et *E. shearii* sont dominantes, ou sont de dominance alternée, selon le mois d'échantillonnage. Elles sont présentes dans 95-100% des échantillons. Dans les 2 sites, la similarité entre les taxocènes mensuels et le taxocène résultant de la somme des isolements de tous les échantillons est d'environ 80-90% . L'organisation des 2 taxocènes, basée sur la fréquence et l'abondance des espèces, est stable, malgré l'augmentation du nombre d'échantillons au cours des différentes saisons.

KEY WORDS : soil fungi, taxocene, *Penicillium*, *Eupenicillium*, *Talaromyces*.

INTRODUCTION

Penicillium species constitute an important part of fungal soils communities. It is a commonly encountered genus represented by many species in forest soils, while they exhibit an irregular distribution in other ecosystems (Christensen, 1981). Otherwise she also noted that its teleomorphs are unfrequent and have an unpatterned distribution.

The 57.4% of isolates obtained from the A_1 horizon of 2 grazing land soils (unpublished data) corresponds to *Penicillium* and its related teleomorphs species. We consider that this part of the community may be treated as a taxocene since the species are likely to be of about the same size, to have similar life histories and compete over the ecological time for a finite amount of similar resources (Hulbert, 1971; Legendre & Legendre, 1979; Gochenaur, 1984; Wicklow, 1985). In addition Hulbert (1971) points out that certain ecological parameters will probably assume a more definite significance when calculated on a taxocene.

On the other hand it has been noted that there is no agreement as regards the temporal variation in soil microfungi. Widden (1986) found no clear seasonal trends in fungal communities, while Gochenaur (1978) observed a strong response of fungal propagules density to seasonal changes.

The aim of this paper is : a) to compare the composition and organization of the *Penicillium* and its teleomorphs taxocene of 2 proximate grazing land soils, which differ in their physicochemical characteristics and usage and b) to establish the seasonal effects in those taxocene's parameters.

STUDY AREA

The study area is located in Canelones Department, north of Montevideo (Uruguay) on Highway 11, 34°40′ latitude south and 55°45′ longitude west. The selected sites are situated on high slopes with 8% gradient, approximately. Both sites are submitted to grazing, with greater intensity at the so called site A (a slope with northern exposure) than at site B (with southern exposure).

The climate is temperate humid. Figure 1 shows the distribution of rainfall and the monthly mean temperature during the year of sampling (September 1985-June 1986). The total rainfall during these months was 1105.8mm.

The soil of site A is an ochric distric argisol (Altamirano & al., 1976) with an A_1 horizon 24cm thick, a silt loam texture and an organic matter content of 2.33%. It exhibits a very slow permeable clayish horizon which saturates periodically with water owing to which it undergoes intense fluctuations in the hidric regime. The soil of site B is a luvic subeutric brunosol (Altamirano & al., 1976) with an A_1 horizon of 21cm depth, a silt-loam texture and an organic matter content of 5.02%. Their physicochemical characteristics are shown in Table 1. Although these 2 soils belong to different large groups, they are related through their texture family and their geological origin.

These soils have been cropped for over 100 years, and at the moment they support a sub-spontaneous pasture vegetation. The vegetation at site A is primarily made up of summer perennial gramineae (with dominance of *Aristida muri-*

na, *Leptocoryphium lanatum* and *Bothriochloa laguroides*) with some winter perennials (dominant: *Piptochaetium montevidense*) and weeds (*Richardia stellaris* and *Evolvulus sericeus*, as dominants). The vegetation at site B is similar to the foregoing, with a greater abundance of *Eryngium horridum* and *Baccharis* spp. (May & al., 1983).

MATERIALS AND METHODS

At both sites a transect was done along which 10 samples were taken at 8m intervals. The decision of analyze 10 samples was based on the results of a preliminary sampling done in July 1985. By means of the increment curve of species it was manifest that although in each new sample there appeared species that were not present in the preceding sample, the curve became asymptotic starting from 8th sample. At each site the vegetation was removed over an approximate surface of 400cm² with a scalpel sterilized in 96% alcohol. The samples of the first 5cm of the A_1 horizon were obtained by means of a sampling cylinder washed with water and 96% alcohol following each extraction. Four plugs were mixed in a plastic bag until a uniform sample was obtained (Christensen, 1981). These were immediately carried to the laboratory and preserved at 5°C until

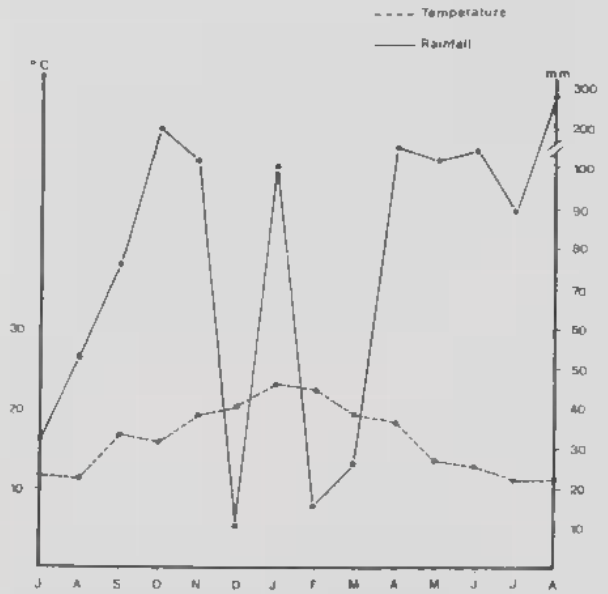

Figure 1 - Rainfall and temperature distribution during September 1985 - June 1986.

Figure 1 - Distribution de la précipitation et de la température pendant la période Septembre 1985 - Juin 1986.

	SITE A	SITE B
Depth (cm.)	0-24	0-21
Texture	silt-loam	silt-loam
pH H_2O	5.7	5.6
pH KClN	4.2	4.1
% Organic matter[1]	2.33	5.02
Available carbon[2]	1.35	2.91
C/N	12.3	13.2
Ca m.e./100g.[3]	3.5	4.9
Mg m.e./100g.[4]	1.6	1.8
K m.e./100g.[5]	0.4	0.4
Na m.e./100g.[6]	0.8	0.5
C.E.C.[7] pH 7.0	9.0	13.3
% saturation of bases	70.0	57.1

[1]Walkley A., 1947 (Walkley-Black Method). [2]Idem [3]Heald W.R., 1965 (68-3.2.3 Method). [4]Id.(68-3.2.4 Method). [5]Soil Conservation Service, 1972 (6Q 2a Method). [6]Id.(6P 2a Method). [7]Id.(5A 1a Method).

Table 1 - Physicochemical properties of soils.
Tableau 1 - Propriétés physico-chimiques des sols.

their processing within 24h. Collection was done in September, December (1985) and March, June (1986).

The dilution plate technique was carried out starting from 10g of dry soil in 90ml of sterile distilled water. One milliliter of 10^{-3} dilution, selected from the preliminary analysis, was distributed over an agar-malt medium (12.5%). The pH was adjusted at 4.5, streptomycin being added at final concentration of 30 ppm to restrain bacteria development. Ten replicates per sample were done. The dishes were incubated in a stove at 20°C. As the colonies emerged they were numbered successively. Colonies that presented the same macro-morphologic features (texture, color, border, zonation, diameter) and the same reaction in the culture media, were ascribed the same number (Gochenaur, 1978).

Penicillium species were identified according to the methodology proposed by Raper & Thom (1949) and corroborated by Pitt (1979). *Eupenicillium* and *Talaromyces* species were also identified by Pitt's methodology. The density and frequency of the species were calculated. The population resulting from each month were compared using Sorensen's Index of Similarity modified according to IS = 2C/A + B, where A is the sum of the relative density plus the frequency for species from one month's sampling, B is the sum for species from the other month and C is the sum of the smaller values for shared species (Gochenaur, 1984). Likewise compared were the populations of each sampling with the summation of the monthly isolates of each species.

The coverage of the species was calculated using Moore and Holdeman's Index: 1 - (n° of the taxa observed once total n° of isolates) x 100 (Gochenaur, 1984). Therefore it was possible to determine the proportion of species represented by one single isolate and hence the probability that a new isolate be an unrecorded species.

RESULTS

From 80 soil samples collected during the study, there were obtained 24 889 isolates belonging to 16 species of the genus *Penicillium* and its teleomorphs *Eupenicillium* and *Talaromyces*. They represent 57.4% of the total fungal isolates (unpublished data).

Site A

The composition and organization of the taxocene was rendered evident through the analysis of 8298 isolates (43.4% of the total isolates of this site) which represented 15 taxa (Tab. 2). *Eupenicillium brefeldianum* was present in all the samples. This species, of such high frequency (100%) and density (77.55%), constitute the dominant member of the taxocene. *Penicillium simplicissimum* was also present in all samples but with a much lower density (14.4%). It was therefore considered as a minor species. Another set of minor species (*P. pulvillorum*[1], *P. pinophilum*, *T. flavus*) occurred in approximately 50% of the samples and they together constitute 5.6% of the total isolates. The remaining 10 species were isolated sporadically with densities below 1%, three of them being solely represented by 1 isolate. The frequency of these rare species did not exceed 25%, except *P. janthinellum* which somewhat exceeded this rate.

The similarity between the *Penicillia* populations of each month and the taxocene resulting from the summation of the 4 samplings ranges from 77.25% in December to 84.77% in September. In every case the Raunkier pattern (Gochenaur, 1978), the dominant species and the minor species with high frequency are maintained. The populations of the 4 months are also highly similar between them (Tab. 3).

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(1) Pitt (1979) contends that *P. pulvillorum* is synonymous of *P. simplicissimum* for they present the same microscopic characteristics. However in this paper they are considered as distinct species. The presence of sclerotia in *P. pulvillorum* is regarded as an important characteristic as it is enabled a reproductive strategy which is not possessed by *P. simplicissimum*.

	SITE A										SITE B									
	SEP.		DEC.		MAR.		JUN.		TOTAL		SEP.		DEC.		MAR.		JUN.		TOTAL	
	RD	F	RD	F	RD	F	RD	F	RD	F	RD	F	RD	F	RD	F	RD	F	RD	F
Eupenicillium brefeldianum (B. Dodge) Stolk & Scott	83.67	100	85.54	100	67.09	100	72.09	100	77.55	100	43.02	100	75.50	100	69.68	100	20.11	100	51.15	100
Eupenicillium shearii Stolk & Scott	0.24	10					2.70	80	0.93	22.5	33.72	100	8.01	100	1.59	80	51.56	100	24.23	95
Penicillium canescens Sopp					0.07	10			0.01	2.5										
Penicillium citrinum Thom							0.80	30	0.25	7.5										
Penicillium expansum Link ex Gray			0.23	30			0.04	10	0.06	10										
Penicillium glabrum (Wehmer)Westling							0.08	10	0.02	2.5										
Penicillium janczewskii Zaleski											1.06	60							0.27	15
Penicillium janthinellum Biourge	1.89	80					1.22	70	0.95	37.5	2.70	80	0.03	10			0.58	40	0.85	325
Penicillium pinophilum Hedgcock	0.40	20	1.25	60	3.37	50	2.17	60	1.65	47.5	0.07	10	0.48	60	0.07	20	0.16	40	0.18	325
Penicillium pulvillorum Turfitt	1.09	50	0.06	10	2.25	60	3.04	100	1.69	55	3.90	60	4.19	90	7.94	90	7.72	90	6.04	825
Penicillium purpurogenum Stoll					0.07	10			0.01	2.5										
Penicillium simplicissimum (Oudem.) Thom	9.85	100	12.81	100	19.79	100	16.92	100	14.42	100	9.63	100	6.42	100	14.42	100	17.19	100	12.21	100
Penicillium verruculosum Dierckx					0.28	20			0.05	5	3.76	80	5.23	90	3.33	90	0.76	80	3.18	85
Penicillium sp.					0.35	10			0.06	2.5										
Talaromyces flavus (Klöcker) Stolk & Samson	2.82	70	0.11	10	6.74	80	0.95	30	2.32	47.5	2.14	80	0.11	10	2.97	90	1.92	80	1.87	65
Talaromyces trachyspermus (Shear) Stolk & Samson	0.04	10							0.01	2.5			0.03	10					0.01	25

Table 2 - Relative density [RD = (n° of isolation of a species /total n° of isolations) x 100] and frequency [F = (n° of samples in which a species was isolated / total n° of samples) x 100] of the species in the *Penicillium* and its teleomorphs taxocene.

Tableau 2 - Densité relative [RD = (n° d'isolements d'une espèce / n° total d'isolements) x 100] et fréquence [F = n° d'échantillons où une espèce a été isolée / n° total d'échantillons) x 100] des espèces du taxocène *Penicillium* et leurs téléomorphes.

S I T E A

	SEP.	DEC.	MAR.	JUN	TOT.
SEP.		70.33	67.62	84.70	84.77
DEC.			74.41	68.43	77.25
MAR.				70.01	82.91
JUN.					81.86
TOT.					

S I T E B

	SEP.	DEC.	MAR.	JUN.	TOT.
SEP.		77.02	79.77	85.42	87.93
DEC.			87.72	80.02	87.35
MAR.				69.04	89.01
JUN.					89.84
TOT.					

Table 3 - Index of similarity among the populations of September, December, March and June and each one with the taxocene resulting from the summation of all soil samples.

Tableau 3 - Coefficient de similitude entre les populations de Septembre, Décembre, Mars et Juin et entre chacun d'eux et le taxocène résultant de la somme des isolements de tous les échantillons de sol.

In this soil only 3 species were represented by one single isolate, that is, the coverage of the remaining species is 99.96%. This means that the probability that a new species will be obtained in a subsequent isolate is 1 in 2000.

Site B

Herein the taxocene parameters were revealed through the analysis of 16 591 isolates (68.4% of all isolates) which represented 10 species (Tab. 2). *E. shearii* and *E. brefeldianum* accounted for over 75% of the isolates and were found between 95% *(E. shearii)* and 100% *(E. brefeldianum)* of the samples. They codominate or alternate their dominance according to the sampling month. A set of 4 minor species *(P. simplicissimum, P. verruculosum, P. pulvillorum* and *T. flavus)* are present between 65% and 100% of the samples but with low densities (between 1.87% and 12.21%) and together represent 23.3% of isolates. The remaining species *(P. janthinellum, P. janczewskii, P. pinophilum* and *T. trachyspermus)* which do not exceed 1% density and 35% frequency, constitute the rare species.

The similarity between the populations of each month and the resulting from the summation of all the samples is very high, nearly 90% (Tab. 3). This reflects a high similarity in composition and organization between them. Nevertheless the 2 species of greatest density only codominate in September since *E. brefeldianum* is dominant in December and March and *E. shaerii* in June.

On the other hand at least 3 of the 4 minor species are present in all the samplings. Of the rare species only 1 was isolated once; hence the probability that a new species will be obtained in a subsequent isolate is 1 in 10000, while the coverage of the remaining species is 99.99%.

The similarity indexes between the populations of the 4 months range from 69% (March-June) to 87.7% (December-March).

DISCUSSION

As the data show, *Eupenicillium* is the characteristic genus of both taxocene analyzed (43.9% of total fungal isolates). This dominance does not agree with findings in other grassland soils in which the dilution plate method was likewise used (Christensen, 1981). On the other hand *Penicillium* spp. accounted for nearly 12% of all fungal isolates. This proportion is slightly lower than those recorded for several grassland soils of the United States (Clarke & Christensen, 1981). *Talaromyces,* an unfrequent genus in grassland soils (Domsch & al., 1980), represents only the 1% of all isolates.

From the analysis of the taxocene of both sites some similarities and differences arise: 1) at site A the distribution of the species follows distinctly the Raunkier pattern (Gochenaur, 1978); so it does at B but with fewer rare species. Hence at site B, 5 times more isolates are needed than at A for a new species to appear. 2) a high similarity index was observed between both taxocenes (73.13%). Nevertheless the number of isolates at site B duplicates that of site A. 3) *E. brefeldianum* is the dominant species at site A and codominates at site B with *E. shearii* (rare at site A). Three of the 4 minor species are the same at both

sites although they exhibit a higher frequency at site B than at site A. The remaining minor species at site A *(P. pinophilum)* is rare at B; conversely, *P. verruculosum*, the remaining minor species at site B, is rare at A.

The organization of the taxocene based on the arrangement of the abundance and frequency of its components is not altered by the repeated sampling in different seasons. The high similarity indexes are due to the presence, in most samples, of one same combination of a species of high density and frequency and one or several minor species with high or low frequency. However it should be noted that at site B *E. brefeldianum* and *E. shearii* exhibit seasonal fluctuations. The abundance of the latter species decreases during the warmest months, and under these conditions there is dominance of *E. brefeldianum*. It is possible to assume that this factor may reduce, directly or indirectly, the abundance of propagules of *E. shearii* in the soil. At site A, *E. brefeldianum* does not exhibit the fluctuations observed at site B, both under the same climate conditions.

The composition of the taxocene changes if the number of samplings increases: the larger the number of samples analysed, the greater the number of rare species isolated, but the rare species do not characterize a taxocene. So, from the ecological standpoint, a repeated sampling is not needed in view that the main ecological parameters are not altered.

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